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SPECIES CONCEPT IN *PENICILLIUM* BASED ON MORPHOLOGICAL CHARACTERS

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SUMMARY. — A recommendation that morphological characters should be employed in the first instance when identifying species of *Penicillium* is presented here. This of itself demands the greatest care in valuating criteria, particularly assuming their range of variation. Morphological criteria may be selected from all stages of the life cycle of the fungus. Their value in the definition of the species will depend upon their constancy and upon the accuracy with which they can be described in qualitative and quantitative terms. The definition of a *Penicillium* species will depend upon the valuation of the sum of their morphological characteristics and upon the recognition of valid levels of differences in criteria selected as definitive. Criteria should show sufficient degrees of differences and possess a considerable measure of constancy to allow effective separation of species. The ranges of variation within the criterion should be known. Ideally, criteria should be obtained from the whole body structures of individuals but because of the complexity, and actual gaps of many life cycles, this is not always possible.

RÉSUMÉ. — L'auteur recommande l'emploi prioritaire des caractères morphologiques lors de l'identification des espèces de *Penicillium*. Ceci requiert le plus grand soin dans l'évaluation des critères, spécialement en précisant leur degré de variation. Les critères morphologiques peuvent être choisis parmi toutes les phases du cycle biologique du champignon. Leur valeur dans la définition d'une espèce dépend de leur stabilité et de l'exactitude avec laquelle ils peuvent être décrits en termes tant qualitatifs que quantitatifs. La définition d'une espèce de *Penicillium* dépendra donc de l'évaluation de la totalité de ses caractères morphologiques et de la reconnaissance des niveaux de validité des différences entre les critères choisis comme définitifs. Ces critères devront montrer des degrés de différence suffisants et présenter un grand degré de constance afin de permettre une séparation réelle des espèces. Mais la complexité et les lacunes existant dans nombre de cycles biologiques, ne permettent pas toujours de parvenir à ce but.

KEY WORDS : *Penicillium*, species concept, morphology.

Charles DARWIN (1859) wrote in his «Origin of species»... : «I look at the term species as one arbitrarily given for the sake of convenience to set of individuals closely resembling each other».

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Similarly, some taxonomists regard species as arbitrarily defined, artificial categories, without existence in nature; but other taxonomists believe that species have an objective reality; that organisms are not abstract species. Instead, with the help of morphological methods, there have been demonstrated completely real groups or organisms, differing between themselves within the limits of each genus by conditions of life (the habitat occupied by them) and also according to the totality of their specific adaptation to these conditions.

It is well known that when a group of organisms reproduces sexually, those classified as belonging to the same species can usually interbreed and produce fully fertile offspring; but those assigned to different species either cannot be cross at all or, if they can, produce infertile offspring such as the case of mules, for example. These observations have led to the notion that interfertility can be a test of whether two individuals belong to the same species, thence the definition of species by MAYR (1965) «as a group of actually of potentially interbreeding natural populations, which are reproductively isolated from other such groups».

Now, what constitutes a species of *Penicillium* ? No answer will satisfy all requirements. The species concept in fungi is a very delicate problem. This is certainly to a large extent due to a very limited use of general characters to test the stability of the morphological criteria employed. That these criteria should be morphological for the vast majority of fungi cannot be disputed. From the standpoint of the mycologists, two or more organisms may be regarded as belonging to the same species if they have the same morphology, including both cultural and microscopical characteristics. However, it must never be forgotten that the morphology of an individual is the ultimate expression of its growth processes, the final display of all its complex relationships with its normal habitat.

Taxonomy is fundamental to all branches of biology that are condensed by sound «natural» classification of the nature of the organisms to be classified and on detailed knowledge of their morphology, physiology, life history, and ecology. Classifications, therefore, develop towards greater stability and become more informative as knowledge of any particular group of organisms deepens.

The modern taxonomic approach in mycology has constantly emphasized the importance of developmental criteria and the necessity of discovering the basic genotypic characteristics of the individual, recognizing that the kinds of substrate on which the fungus grows and the habitat conditions to which it is exposed result in different phenotypic expressions of the genotype. Emphasis has been placed on a search for these criteria less subject to pressures arising from differences in substrate or habitat. But the definition of valid taxa must take into account as wide a range of criteria as possible and the valuation of the degree of variation which may be expected of each criterion.

In nature, the majority of fungi, with the exception of obligate parasites, live in communities of organisms exposed to competition pressures which of necessity have a limited effect upon the degree of expression of their genotypic potentialities. For those fungi easily cultivated on laboratory media, which is

the case of *Penicillia*, freedom from competition pressures allows the individual to express other aspects of its genotype, to display different morphological criteria or at least different degrees of already recognized criteria. This of itself demands of the taxonomist who must attempt to define the species from laboratory cultures the greatest care in valuating criteria, particularly with respect to assessing their range of variation : an agar medium not only differs from the majority of natural substrates in chemical composition, but also possesses entirely different physical properties. But these criteria cannot stand alone in the control of taxa, particularly at the specific level; where possible they should be referred to a final court of appeal before a name is assigned, to the authentic herbarium material lodged by the author of a species name. Equally for the new species, published descriptions, illustrations, and measurements should be supplemented by dried specimens lodged in permanent herbaria. Live cultures of *Penicillium* properly named, have their uses as references, but a fungus relieved of the pressures of its native habitat often becomes attenuated, partly because of the unnatural conditions of pure cultures, partly because of its intrinsic variability, and partly because of the hazards of transfer techniques.

Morphological criteria may be selected from all stages of the life cycle of the fungus. Their value in the definition of the species depends upon their constancy and upon the accuracy with which they can be described in qualitative and quantitative terms. If a fungus can be cultivated on laboratory media, the morphology of its mycelium, its branching, the degree of constancy of septation, and measurement of hyphal width, can all be obtained with precision and are valid for that individual medium at that time. They are valuable so long as it is realized that they refer to a particular individual under a defined set of culture conditions and their value increases with the accuracy of definition of these conditions and with comparative studies of their constancy under related conditions for the same individual and for closely related individuals.

The definition of *Penicillium* species depends upon the valuation of the sum of their morphological characteristics and upon the recognition of valid levels of differences in criteria selected as definitive. Not all criteria are of equal value at the same level of taxonomic separation. Spore producing members and the stage of development through which they pass, and the spores which they bear, are more constant than many of the characteristics of the mycelium that produced them.

The important requirements demanded of morphological criteria for taxonomic purposes may be stated as follows :

1) Criteria should show sufficient degrees of differences and possess a considerable measure of constancy to allow effective separation of species.

2) Ranges of variation within a criterion should be known.

3) Within limits, each criterion should be capable of being observed accurately.

Ideally, criteria should be obtained from the whole body structures of individuals but, because of the complexity and actual gaps of many life cycles, this

is not always possible.

While mycologists faced with the identification of fungi must applaud the increase in morphological data that can be used to separate species, they must be constantly aware of the frustrations implicit in much of this knowledge.

Taxonomy of living organisms cannot remain static as the limits of knowledge continually expand, but the very rate of expansion brings problems in its wake because much of the knowledge concerns individuals and can be accounted of real value only when the volume of it allows generalization on a sound firm basis.

It is customary to select morphological criteria from consideration of the several distinctive features of individuals. For *Penicillium* species this should involve a consideration of the structure of the conidiophore and its several parts of the way in which it reaches its mature form. It must also involve the consideration of any sporing structure which the conidiophores produces, their development and final anatomical structure, and the origin and morphology of the spores which they carry. While the conidiophore can show marked changes in relation to the environment, sporing structures remain remarkably constant for every individual included in a single species concept. The criteria derived from the morphology of the spore producing members constitute, thus the major factor for the separation of *Penicillium* species.

Where an individual can be cultivated on laboratory media, the difficulties inherent in the close relationship of the mycelial hyphae with the natural substrate are avoided and hyphal morphology can be examined on and in the mycelium. But the value of mycelial data requires careful scrutiny, not only with respect to its precise taxonomic status but also with respect to the degree of accuracy with which such information can be communicated to other investigators.

While it is possible to relate mycelial colour and any pigment changes produced in the culture media to standard colour charts, it is not always possible to ensure that two observers match colour values with the same degree of accuracy. When it comes to the description of the gross appearance of the colony, difficulties are magnified because of a lack of precise terms to describe the form and colour of colony, hence the use of colour photographs by myself in my identifications. The advent of colour photography spread a ray of hope, although the cost of reproduction has proved prohibitive. However, colour reproductions have much to recommend them as worthy of confidence where differences in gross colony characters are really essential for a definition of a species.

By contrast, measurements of hyphal diameters, degree of branching, angles of divergence of branches, and intervals between primary branches and secondary ones may be combined with the information on the constancy and degree of septation, the occurrence of particular cell shapes, and all can be accurately recorded and illustrated. Insofar as mycelium is concerned, it is the detail of its component parts which is more important than the gross characters of the whole, but even these criteria must be used with care and their use must be accompanied by detailed facts about the conditions under which they were observed.

One of the great difficulties in tracing the borderlines between species closely related is the variability of *Penicillium* species, derived from the incredible capacity of the genetic endowment to generate random variations which are either conserved or eliminated under the selective pressures imposed by the environment.

The *Penicillia* indeed represent an unusually variable group of moulds, and actually, no mycologist needs to be told that any species of fungus is highly variable. Polygenic systems are rather prominent as a major basis for variability, and this is to be expected in haploid organisms lacking a diploid basis of heterosis. Although successful taxonomy and nomenclature must be based upon strains considered to be «normal», the mycologist must recognize that variations and mutations often occur. In laboratory cultures, these usually arise in one or two ways. They may develop as a result of progressive change under continued laboratory cultivation and appear as sectors or areas of altered colouration, colony texture, or rate or habit of growth. On the other hand, they may appear as limited sectors or areas showing an abrupt change in some conspicuous characters such as colour of ripe conidia or an inability to develop normal conidial structures upon the substratum employed. The latter type of change commonly remains stable through subsequent recultivation, hence may be regarded as a true mutation; the former type often continues to show further change in the same or in some other direction, hence is usually regarded as representing a sort of step in a process of progressive variation. Strains believed to represent both types of development may be isolated from natural sources. If reason exist for believing them to represent merely different aspects assumed by a common and cosmopolitan species, such variations or mutations are usually not accorded taxonomic status.

Culture appearance and morphology must then be expected to vary within certain range, governed by limits established for the species, and these limits should represent the product of observation, experience, and good judgement. In any elaborate study of a particular species, the range of variation in structure is important.

SPECIES DEFINITION

A definition of species, the unit of classification, is hard to find. As a matter of fact, definitions will inevitably vary with individual opinion.

COWAN (1965) defined Taxonomy as composed of three parts :

1) Classification,
2) Nomenclature, and
3) Identification of the unknown.

Nomenclature, which is the least important part, has its Code and Rules, whereas classification, the most important part of Taxonomy has nothing.

In my opinion, a definition of microbial species that was first published by GORDON & MIHN (1962) could be a good starting point to reach an agreement

on a definition of species. Currently the definition is as follows :

«A microbial species is a concept represented by a group of strains from a variety of sources, or by a population of strains, that contain freshly isolated strains, stock strains maintained in collections for varying periods of time, and their variants (strains not identical with their parents in all characteristics) which have in common a set of correlating stable properties that separate the group from the other groups of strains».

To delineate a species according to this definition, therefore one must study strains from a variety of sources and include newly isolated strains, old stock strains, and as many as their variants as possible; select the characteristics these strains have in common for describing the species they represent; and stress the similarities of all these strains, not their differences.

Now we ask : why include old stock strain ?

1) Our nomenclature is tied to old strains or to strains that are rapidly getting older because the first or one of the first strains given a new species name by an author becomes the nomenclatural type strain of the species. The nomenclatural type strain must always belong to the species and be recognizable from the species description. In applying new tests and observations that result in the emendation of a species description, therefore one must include the nomenclatural type strain.

2) The old stock strains are also important in taxonomy because they represent the history of taxonomy of the work of a taxonomist who preceded us, and history forms an important part of every branch of knowledge and experience.

3) The most important reason, however, for including old strains in the taxonomic study of a species is the relative stability of their characteristics as compared with those of the characteristics of newly isolated strains.

Fungal strains possess many properties of varying degrees of stability. In our own experience, characteristics that persist after years of cultivation in test tubes are the most stable characteristics.

Why are variants (adapted strains and mutants) incluedc in the present definition ?

Variants, developed in nature or in the laboratory, are part of a population of strains, and because they are there, they have as much right to a species name as the nomenclatural type or the newest isolate from nature. Variants as well as old strains, can contribute to the determination of the more stable properties of the species. The selection of properties common to new isolates, old strains, and their variants will result in a more reliable description of the species.

Fungal variation and the spectrum-like relationship of the strains have to be kept in mind as two of the facts of life. We can compensate for these by including many correlating properties of the strain as possible in the distinctive set, or pattern, used to delineate the species. The distinguishing pattern of correlating properties must be large enough to permit variations by a strain in a few properties (one, two, or three, perhaps) without excluding the strain from the

species; that is, despite some variation, the overall pattern is that of the species. It is the similarities of the strains that are stressed rather than their differences.

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